A Thesis for the Degree of Master of Science

Emulsion gels stabilized by octenyl succinic anhydride-modified pregelatinized starch

옥테닐호박산-알파전분으로 안정화된 에멀션 젤

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Department of Agricultural Biotechnology

Seoul National University

Chang, Min Jea

석사학위논문

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옥테닐호박산-알파전분으로 안정화된 에멀션 젤

지도교수 최영진

이 논문을 석사학위 논문으로 제출함

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장 민 제

장민제의 석사 학위논문을 인준함

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위원장 하남출 (인)

부위원장 최영진 (인)

위원 장판식 (인)

Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The aim of this study was to prepare and to investigate physicochemical characteristics of OSA-modified pregelatinized starch (O-PGS) and emulsion gels stabilized by O-PGS. To prepare O-PGS, OSA-modification was conducted on gelatinized maize starch and ethanol precipitation and freeze drying was carried out. The crystalline structure of PGS with ethanol precipitation was quite different from original starch granules and OSA-modification had no effect on crystalline structure of PGS. DSC thermogram showed that PGS and O-PGS was cold-water soluble. FT-IR data confirmed that OSA-modification was conducted successfully and maintained throughout ethanol precipitation and lyophilization step. After preparation of emulsion gels, oil-water interface was stabilized by O-PGS and the oil droplets were entrapped in starch network matrix. Emulsion gels showed typical pseudoplastic behaviors. Higher concentration of O-PGS and oil phase led to increase in apparent viscosity and pseudoplastic characteristics in emulsion gels. In frequency sweep tests, emulsion gels had higher storage modulus and loss modulus by increasing O-PGS and oil concentration, and showed the characteristics of viscoelastic fluids in lower and gel-like

properties in higher concentration of O-PGS and oil phase. Emulsion gels prepared by different concentration of oil phase and O-PGS showed wide range of rheological properties. As a result, O-PGS can be applied into foods that have various kinds of rheological properties.

Keywords: Emulsion gel, OSA-starch, pregelatinized starch, hydrogel, emulsion rheology.

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I. INTRODUCTION

In food industry, food products including desserts, dressings, and sauces have gel-like structures (Mun, Kim, Shin, & McClements, 2015). Gels are composed of three-dimensional cross-linked polymer network in the liquid phase so they have solid-like behaviors (Ferry & Ferry, 1980). Gel structure is widely used in current food industry because of its thermodynamic stability and consumer preferences (Geremias-Andrade, Souki, Moraes, & Pinho, 2016).

Oil-in-water emulsions consist of lipid droplets stabilized by emulsifier that dispersed in continuous aqueous phase. Emulsifiers can stabilize oil-water interface due to its amphiphilic characteristics. In industry including pharmaceuticals, cosmetics, and foods, emulsions are used widely to contain lipophilic components that are insoluble in water but soluble in oil (Mcclements, 2007, 2015).

Some food products having gel-like characteristics need oil phase to incorporate lipophilic nutraceuticals like polyphenols or phytosterols (Mun et al., 2015). Then, emulsion gel system can be applied to add lipophilic

molecules in these food products. Emulsion gels are defined as soft solids that contains oil droplets entrapped in a gel matrix. Emulsion gels are usually prepared with proteins or polysaccharides that forms three-dimensional structure in continuous phases (Torres, Murray, & Sarkar, 2016). Gel-like structure can provide higher stability on food systems against gravitational separation such as creaming and sedimentation due to its semi-solid structure, and consumer preferences by its own texture.

Common preparation processes of emulsion gels consist of two main steps, emulsion preparation and formation of gel network (Dickinson, 2012; Guo, Ye, Lad, Dalgleish, & Singh, 2014; Torres et al., 2016). Emulsion preparation step includes high-shear blending, ultrasonic treatments, or high-pressure homogenization which are commonly utilized. Hydrogels in emulsion gel usually incorporate polysaccharides or proteins in previous studies about emulsion gels. Many kinds of processes are employed in gel network formation step. Adding gelling agents, salts, or acids in protein-based emulsion gels are applied in former studies. Also, associating emulsions with prepared hydrogels such as starch paste can be used to manufacture emulsion gels. Then, incorporation of two diverse step into one step can enhance the efficiency of processing emulsion gels.

Starches are one of the most abundant component in food industry

because of its biodegradability and low-price. In oil-in-water emulsion system, emulsion stabilizer needs both hydrophobicity and hydrophilicity to stabilize oil-water interface (Fonseca-Florido et al., 2018). However, starches are composed of glucose chains with α-(1→4) linear and α-(1→6) branched glycosidic bonds, and it has hydrophilic characteristics because of many hydroxyl (-OH) functional groups. Due to its hydrophilic characteristics, starches have restricted ability to stabilize oil-water interfaces. Therefore, starches can be modified with octenyl succinic anhydride (OSA) by esterification under alkaline condition to increase hydrophobicity to utilize starch as an emulsifier. OSA-modified starches can stabilize oil-water interface in emulsion system because it has both hydrophobicity and hydrophilicity (Dokić, Krstonošić, & Nikolić, 2012; Krstonošić, Dokić, Nikolić, & Milanović, 2015; Sweedman, Tizzotti, Schäfer, & Gilbert, 2013; Tesch, Gerhards, & Schubert, 2002). Studies that investigated emulsions stabilized with OSA-starch has many forms of starches including dissolved state of OSA-starches (Dokić et al., 2012; Matos, Marefati, Gutiérrez, Wahlgren, & Rayner, 2016), Pickering emulsions with starch granules (Rayner, Sjöö, Timgren, & Dejmek, 2012), starch nanoparticles (Saari, Fuentes, Sjöö, Rayner, & Wahlgren, 2017) and starch nanocrystals (Jo, Ban, Goh, & Choi, 2018). Due to its advantages of biodegradability and low-price, OSA-starch is widely used in food, cosmetics, and pharmaceutical industries.

However, in previous studies, OSA-starch has to be heat treated before emulsion preparation to make dissolved state of OSA-starch, except utilized in Pickering emulsions (Dokić et al., 2012; Krstonošić et al., 2015; C. Li, Fu, Luo, & Huang, 2013; Lin, Liang, Zhong, Ye, & Singh, 2018; Matos et al., 2016). In starch based Pickering emulsions, large droplet sizes can induce creaming of emulsion droplets due to large granule sizes in range of 10-100 μm in droplet diameters (Matos et al., 2016; Rayner et al., 2012). Pre-heating of OSA-starch in preparation of emulsions makes it difficult to apply OSA-starches in industrial fields. Therefore, when making OSA-starch as cold-water soluble, the process of preparing emulsions stabilized by OSA-starch can be simplified. In preparation of emulsion gels with OSA-starch, there should be treated with additional gelling agent or be associated with prepared starch paste with emulsions (Mun et al., 2015; Torres et al., 2016).

OSA-modification can disrupt the interaction between amylose and amylopectin, so OSA-modification led to decrease in viscosity and gelatinization temperature. Due to this characteristics, no study had figure out emulsion gels stabilized by OSA-starch alone. For these characteristics of OSA-modified starch, no literature has investigated the preparation of emulsion gel alone with OSA-starch.

Gelatinization of starches results in swelling of water in starch chain

and loss of crystallinity and birefringence. These effects make pregelatinized starch to be soluble in cold water. Pregelatinized starches are commonly used as thickening agent in food industry, especially for pie fillings, puddings, sauces, and baby foods (Patindol, Shih, Ingber, Champagne, & Boue, 2013). Also, pregelatinized starch can be added to emulsions stabilized by egg yolk or sodium caseinate to increase apparent viscosities of emulsions (Bortnowska, Balejko, Tokarczyk, Romanowska-Osuch, & Krzemińska, 2014). The well-known processes for preparing pregelatinized starch are spray drying (Laovachirasuwan, Peerapattana, Srijesdaruk, Chitropas, & Otsuka, 2010), drum drying (Nakorn, Tongdang, & Sirivongpaisal, 2009), and solvent-based method (Patindol, et al., 2013). Among them, solvent-based method is effective for removing remaining ions after OSA-modification of starch paste than other preparation methods. In this study, OSA-modified pregelatinized starch was adopted to prepare cold-water soluble OSA-starch and emulsions.

Rheological properties of emulsions have important role in emulsions stability and consumer preferences. In emulsion system, gravitational separation including creaming and sedimentation is one of the most common phenomenon that reveals emulsion unstability. The stability against gravitational separation can be enhanced by increasing viscosity of emulsions. Therefore, various thickening agents such as gum arabic, carrageenan, and

pectin were used in food system to improve physical stability of emulsion and consumer preferences. Pregelatinized starch can also be used to enhance viscosity of emulsions and salad dressings in previous studies (Bortnowska, et al., 2014)(Bortnowska et al., 2014).

The aims of this study were to prepare OSA-modified pregelatinized starch (O-PGS) and investigate its characteristics, and utilize O-PGS as an emulsifier to manufacture emulsion gels and investigate its rheological properties, lipid droplet sizes with different concentration of O-PGS and oil phases, and simulated *in vitro* digestion pattern.

Ⅱ. MATERIAL AND METHODS

2.1. Materials

Standard maize starch was provided by Roquette S. A. (Lesterm, France). Octenyl succinic anhydride (OSA), Nile Red and fluorescein sodium salt was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Ethanol was obtaind from Daejung Co. (Korea). Canola oil was purchased from a local supermarket. All chemicals were of reagent grade.

2.2. Preparation of OSA-modified pregelatinized starch

First, 27 g of standard maize starch was dispersed in 900 ml of distilled water and heated in autoclave by 105℃ for 15 min to gelatinize the starch. After cooling the starch paste into room temperature, the pH of starch paste was adjusted into 8.5 with 0.5 M NaOH and OSA (3% of dry starch) mixed with ethanol (5 times diluted) was slowly dropped into starch paste for 3 h. The pH of starch paste was controlled by 0.5 M NaOH in the range of 8.0 to 9.0 during OSA addition. After the reaction, starch paste was neutralized to pH 6.5 by 0.5 M H_2SO_4. When the neutralization was finished, double volume

of ethanol was mixed with starch paste for precipitation and isolation of O-PGS in the solution. The precipitates were centrifuged by 4000 g and washed twice with absolute ethanol, and then freeze dried for 24 h. After freeze drying, O-PGS was stored in room temperature.

2.3. X-ray diffraction (XRD)

The crystalline structures of standard maize starch, PGS, and O-PGS were determined by X-ray diffractometer (Bruker model D8 advance, Karlsruhe, Germany) with Cu kα radiation (λ = 1.54 Å). Reflection angle signals of 2θ from 4° to 40°. The scan speed was 0.5 s per step and Phi rotation was 30 min^{-1}.

2.4. Differential scanning calorimetry (DSC)

The thermal properties of standard maize starch, PGS, and O-PGS solutions were determined by differential scanning calorimeter (Q200, TA Instruments, Delaware, USA). Sample were dispersed in distilled water by 20% (w/v) concentration. Each sample was placed in a hermetic aluminum pan by 10 to 20 mg, and an empty pan was used as a reference in all measurements. The DSC scan started at 25℃, increased by 3℃ min^{-1} to 95℃.

2.5. Fourier transform infrared spectroscopy (FT-IR)

PGS and O-PGS were analyzed by an infrared spectrophotometer (Nicolet 6700, Thermo Scientific, USA) at 25℃ to figure out the esterification between hydroxyl groups in starch and OSA. For each sample, wavenumber range from 650 to 4000 cm^{-1} was scanned at 8 cm^{-1} of resolution over 32 scans.

2.6. Preparation of emulsion gels

5.0, 10.0, 20.0 wt% of canola oil and 0.02% sodium azide solution (aqueous phase) containing certain concentrations (3.0, 5.0, 10.0 wt%) of O-PGS was mixed by high-shear blender (Ultra-Turrax T25D, Ika Werke GmbH & Co., Staufen, Germany) at 15,000 rpm for 2 min in room temperature. Prepared emulsion gels were stored in room temperature.

2.7. Preparation of emulsions with ultrasonic treatment

In applying lower concentration of O-PGS as a stabilizer, 1.5 and 3.0 wt% of O-PGS and 5 wt% of oil phases were homogenized. First, high-shear blender was used at 15,000 rpm for 2 min in room temperature and then ultrasonic treatment was applied by 1, 2, 3, and 5 min.

2.8. Confocal laser scanning microscopy (CLSM)

Emulsion gels were imaged via CLSM to figure out the structure of oil droplets that entrapped in starch gel matrix. Canola oils containing 1% of Nile Red was used before emulsion preparation, and 10 μL of fluorescein isothiocyanate (FITC) was used to stain starch gel matrix. The excitation lines were both 488 nm for Nile Red and FITC and the detection lines were 630 nm for Nile Red and 520 nm for FITC.

2.9. Rheological properties of emulsion gels

2.9.1. Flow behavior of emulsion gels

The rheological properties of emulsion gels were measured by rotational rhemometer (Rheostress RS 1, Haake Instruments, Karlsruhe, Germany) using cone-plate C35/1 Ti sensor with 0.052 mm gap at 25℃. In all measurements, 0.2 ml of emulsion gel was placed on the plate. First, to investigate the flow behavior of emulsion gels with different O-PGS and oil concentration, the samples were exposed 300 s to increasing shear rate from 0 to 500 s^{-1}. The power law (Equation (1)) was used to determine flow behavior pattern where σ is the shear stress (Pa), K is the consistency coefficient, $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the flow behavior index (-).

$$\sigma = K\dot{\gamma}^n$$ Equation (1)

2.9.2. Dynamic viscoelasticity of emulsion gels

To investigate the dynamic viscoelasticity of emulsion gels, oscillatory frequency sweep test was carried out. The changes in storage modulus and loss modulus was measured with a strain of 0.05 ± 0.005 and frequency range was between 0.1 and 10.0 Hz. The storage modulus (G') and loss modulus (G") were calculated from the strain response.

2.10. Measurements of droplet sizes and zeta-potential

Droplet sizes and zeta-potential of emulsions prepared by low concentration of O-PGS (1.5, 3.0 wt%) and ultrasonic treatment were determined by a Zetasizer (Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK) using a helium-neon laser (λ = 633 nm).

2.11. Storage stability of emulsions in different temperatures

Storage stability of emulsions prepared with low concentration of O-PGS and ultrasonic treatment was investigated. Emulsions prepared by 5 min of sonication were stored in 4, 25, and 42℃ and droplet sizes and zeta potentials were measured in day 0, 1, 7, 14, 21, and 28.

2.12. Statistical analysis

Power law regression for flow behavior of emulsion gels and emulsions prepared by ultrasonic treatment were analyzed by Sigmaplot 10.0 (Systat Software, San Jose, CA, USA). These experiments were conducted triplicate.

Ⅲ. RESULTS AND DISCUSSION

3.1. Characterization of O-PGS

3.1.1. Crystallinity of ethanol precipitated PGS and O-PGS

In preparation stage of O-PGS, starch gelatinization, OSA-modification and ethanol precipitation was carried out. The crystalline structure of standard maize starch was collapsed after gelatinization as shown in Figure 1. Peaks on 15.3°, 17.1°, 18.5°, and 23.5° revealed that standard maize starch had typical A-type crystalline structure that has been found widely in cereal-based starches(Altuna, Herrera, & Foresti, 2018). PGS and O-PGS had peak on about 13.5° and 20.8 that exhibits a V-type crystalline structure (Qin, Liu, Jiang, Xiong, & Sun, 2016). Gelatinization of starch occurs when starch solution was heated. Starch gelation is the result of water absorption and swelling of starch granules, followed by leaching of linear amylose chains, and close packing of swollen starch granules (Mun et al., 2015). Starch crystalline structure is composed of packing of amylose helix, but in gelatinization process, it collapses and amylose and amylopectin chains were dispersed and the crystallinity of original starch would be destructed. The interactions between amylose and amylopectin chains in starch paste caused

higher viscosities because it had many hydroxyl bonds(-OH).

In PGS and O-PGS, V-type crystallinity was consisted of inclusion complex of amylose and ethanol. When starch paste was mixed with ethanol, single-helical structure of amylose and ethanol was formed and it exhibited V-type diffraction peaks (Sun, Gong, Li, & Xiong, 2014). (Lee, Yang, Chung, & Lim, 2017) also investigated X-ray diffraction pattern of rice originated pregelatinized starch that demonstrated V-type crystallinity and suggested that V-type structure was due to amylose-lipid complexes by single helices. PGS and O-PGS had peaks on same angles so OSA-modification process had no influences on basic crystalline structure of PGS. Also, previous study suggested that the addition of OSA led to steric repulsion and it disrupted the interaction between amylose and amylopectin (Ettelaie, Holmes, Chen, & Farshchi, 2016).

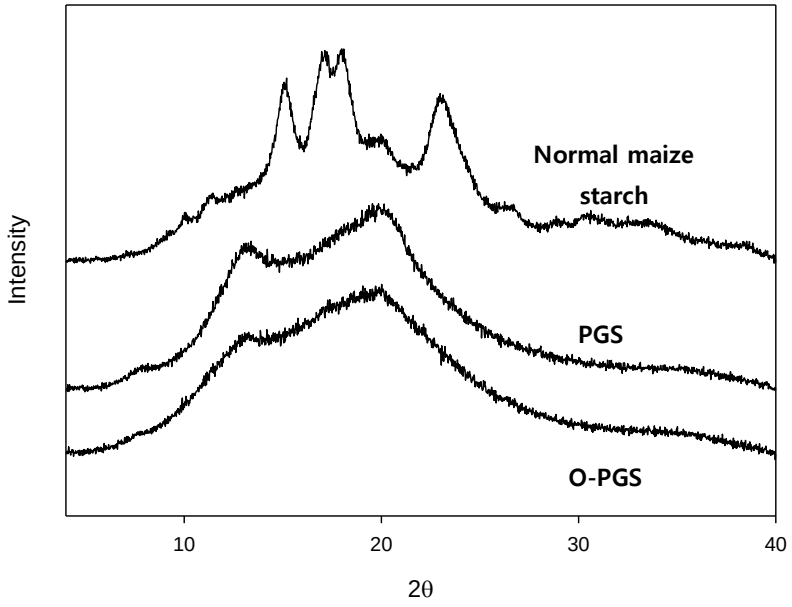

Figure 1. X-ray diffractograph of standard maize starch, PGS, and O-PGS.

3.1.2. Thermal properties of PGS and O-PGS

Ethanol precipitation and freeze drying of starch paste with and without OSA-modification led to pregelatinized state of starch. In Figure. 2, DSC thermogram showed that gelatinization of standard maize starch occurred at about 65℃, but PGS and O-PGS had no peaks on DSC thermograms. In PGS and O-PGS, there was no phase transition by heating from room temperature to 95℃ because it had cold-soluble properties. Similar results were investigated by physically modifying buckwheat starch by preparing pregelatinized starch (W. Li et al., 2014). Previous study suggested that pregelatinized starch could be prepared by precipitation induced by mixing ethanol and starch paste (Patindol, Shih, Ingber, Champagne, & Boue, 2013). In addition, Figure. 2 showed that OSA-modification of starch paste did not influence thermal properties of PGS. Ethanol precipitation formed single-helix inclusion complexes of starch and ethanol which was more easy to be disrupted than original A-type maize starch (Qin et al., 2016). In previous studies, OSA-starch solution needed pre-heating to be dissolved, but our research suggested that O-PGS had no need of pre-heating in emulsification step (Dokić et al., 2012; C. Li et al., 2013; Lin et al., 2018). Emulsions stabilized by OSA-starch without pre-heating can simplify preparing process of emulsion and emulsion gels.

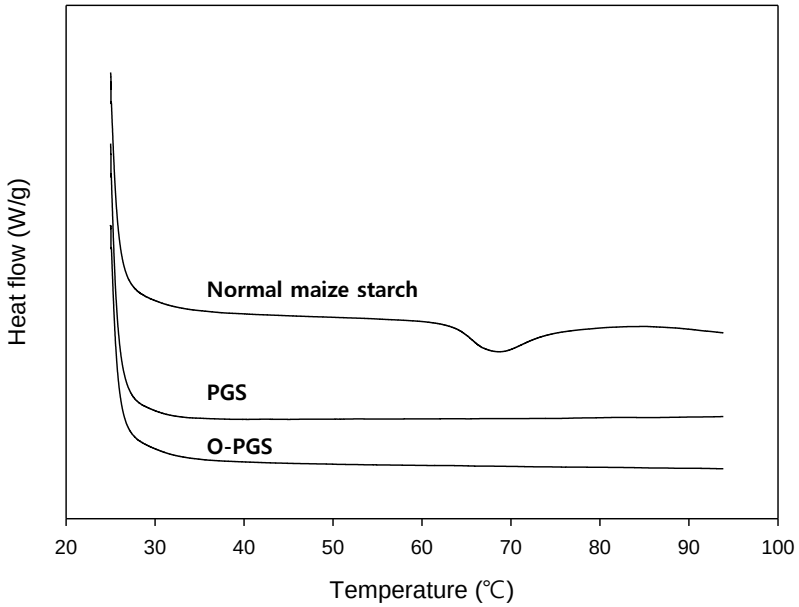

Figure 2. DSC thermograms of standard maize starch, PGS, and O-PGS.

3.1.3. Infrared spectra of O-PGS

The infrared spectra of O-PGS and PGS without OSA-modification showed that the esterification between OSA and starch paste was carried out successfully and it maintained during ethanol precipitation and freeze drying. In O-PGS spectrum, additional bands at 1570 cm^{-1} was revealed that was not existed in PGS spectrum. The band at 1570 cm^{-1} was due to existence of carboxylate RCOO- and this result shows that OSA was added onto hydroxyl group of starch (Fonseca-Florido et al., 2018). Previous studies about OSA-modified starch also confirmed that peaks on 1570 cm^{-1} reveals C=O bond caused by esterification of OSA and hydroxyl group of starches (Ye et al., 2017). OSA-modification provided hydrophobic characteristics on pregelatinized starch so O-PGS could have amphiphilic characteristics to stabilize oil-water interfaces.

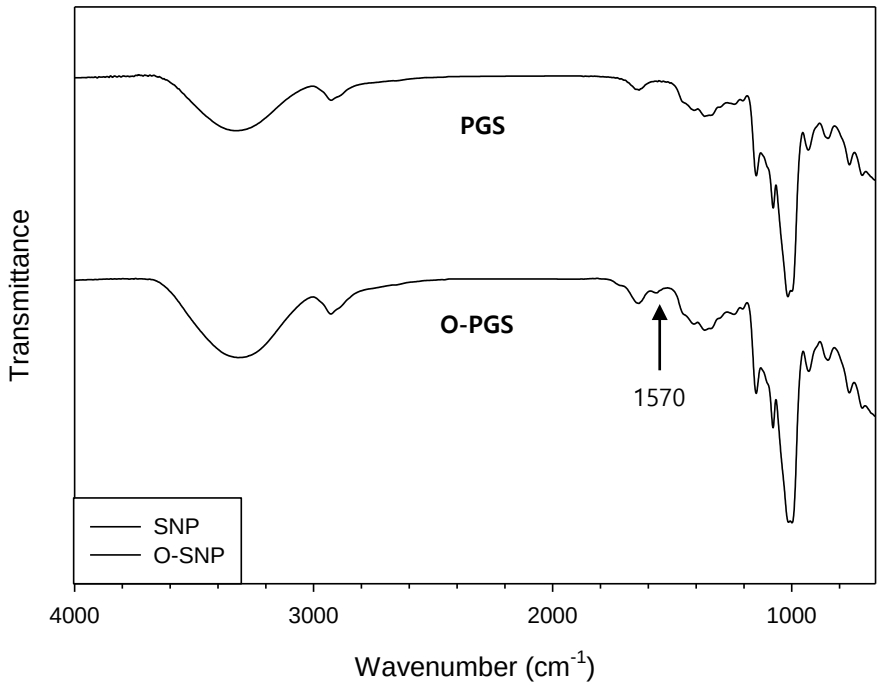

Figure 3. Infrared spectra of O-PGS and PGS.

3.2. Emulsion gel formation

PGS without OSA-modification could not stabilize oil-water interfaces. However, with increased hydrophobicity by OSA-modification, O-PGS could stabilized oil-water interfaces. Oil droplets were stuck into the starch matrix in Figure. 3. When O-PGS was dissolved in aqueous phase with high shear blender, it formed starch gel network. O-PGS had both hydrophilicity and hydrophobicity so it could stabilize oil-water interfaces and oil droplets were entrapped in O-PGS matrix.

Hydrogels containing emulsions were investigated in various previous studies. Many studies prepared emulsion gels with heating, salting, or mixing prepared emulsions with hydrogels. In this study, emulsion gels were manufactured by one-step processing due to the characteristics of pregelatinized starch. When O-PGS was solubilized in water by high-shear blending, it forms starch gel network in continuous phases while stabilizing oil-water interfaces.

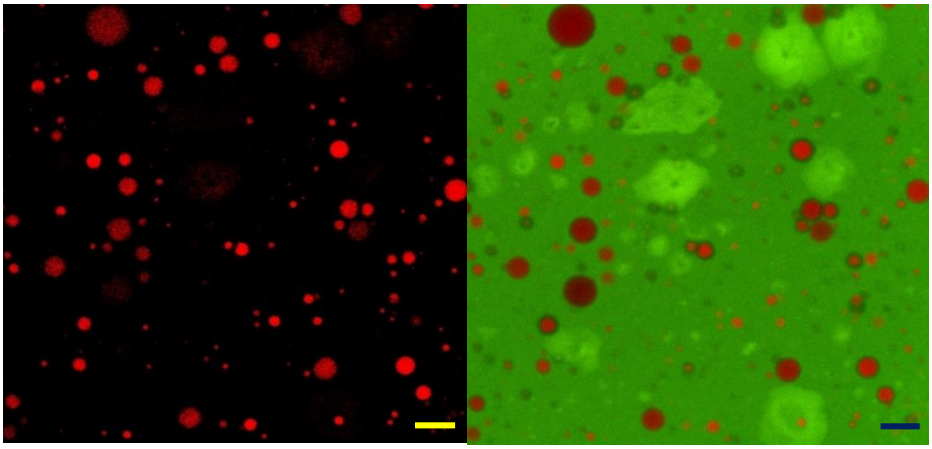

Figure 4. CLSM micrograph of 10 wt% oil-in-water emulsion gel stabilized by 5 wt% O-PGS. Oil droplets were stained by Nile Red and continuous phase was stained by Fluorescein sodium salt. Scale bar represents 10 μm.

3.3. Rheological properties of emulsion gels

3.3.1. Flow behavior of emulsion gels

The rheological properties of the emulsion gels prepared with different concentration of O-PGS and oil phase were investigated by a rheometer. First, the relationship of shear stress and shear rate of emulsion gels showed concave downward curve that demonstrates emulsion gels had typical pseudoplastic behaviors, so called shear thinning behavior in Figure. 5, 6, and 7. In these figures, emulsion gels had higher apparent viscosity when it had higher concentration of O-PGS and oil phases.

Flow behavior of emulsion gels was analyzed by the Power law regression. In Table 1, increase in O-PGS and oil concentration led to increase in consistency coefficient (K) and decrease in flow behavior index (n). Consistency coefficient values (K) reveals apparent viscosities of fluids. Increasing O-PGS concentration led to increase in interactions of amylose and amylopectin in continuous phases in emulsion gels and it caused increase in apparent viscosities and pseudoplastic characteristics of emulsion gels.

Increase in oil concentration also affected consistency coefficient and flow behavior index values. Effect of concentration of oil phases on apparent viscosity and pseudoplastic behavior of emulsion gels was that lipid droplets

bound to O-PGS matrix could act as “active” filler in emulsion gels. Active filler particles entrapped in gel matrix was stabilized by interactions between macromolecules and surfactant molecules in continuous phases while inactive filler particles have no interactions with gel matrix (Geremias-Andrade et al., 2016). Therefore, active filler particles in gel matrix can have effect on rheological properties significantly with amount of concentrations. In our study, lipid droplets were stabilized by amphiphilic O-PGS molecules in starch matrix of emulsion gels. O-PGS acted as both roles of surfactant molecules of lipid droplets and gelling agent of continuous phases, so increasing oil fraction led to increased viscosities.

When comparing to conventional OSA-starch stabilized emulsions with similar concentration of the starch and oil, emulsion gels prepared with O-PGS had much higher consistency coefficient about 10 to 100 times and lower flow behavior index (Dokić et al., 2012). Another study that investigated rheological properties of emulsions stabilized by hydrophobic maize starch also had pseudoplastic characteristics that had decreasing apparent viscosities (Ye et al., 2017).

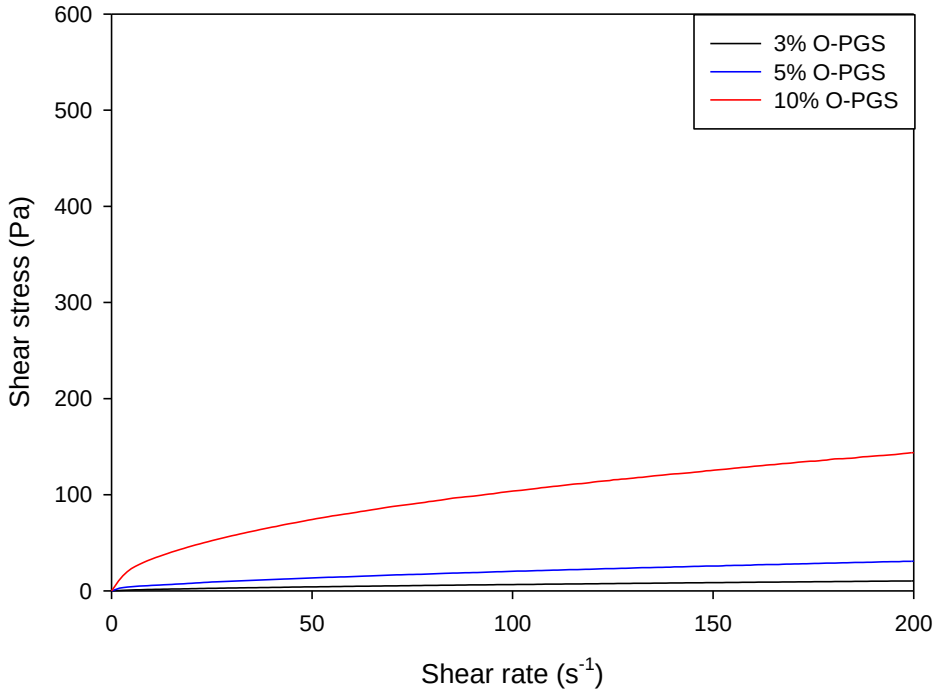

Figure 5. Representative flow curves of emulsion gels with 5 wt% oil and 3, 5, and 10 wt% of O-PGS.

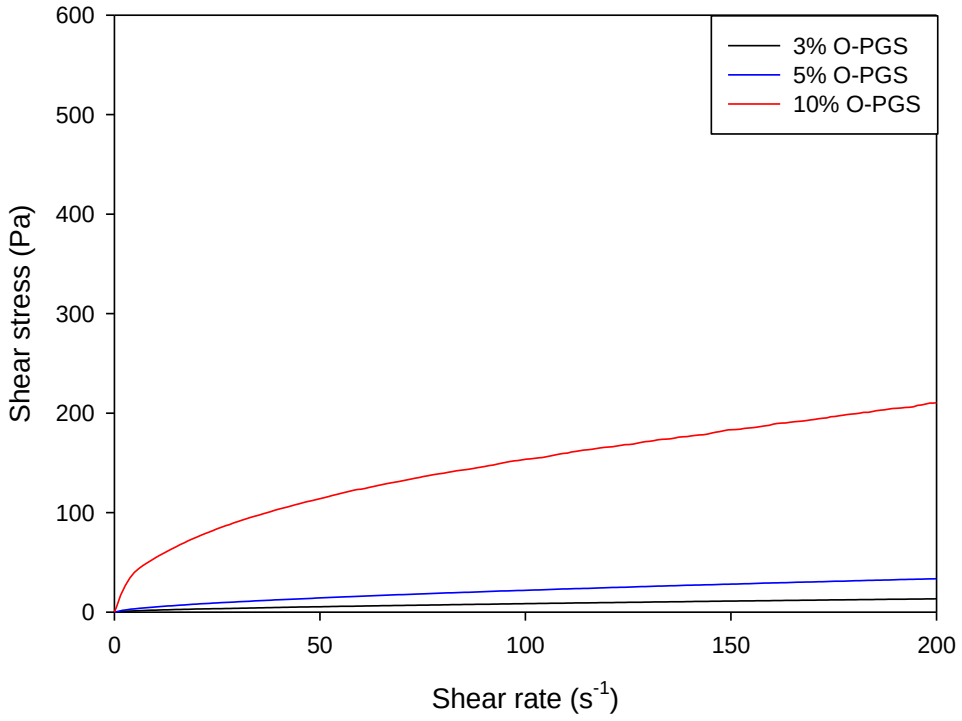

Figure 6. Representative flow curves of emulsion gels with 10 wt% oil and 3, 5, and 10 wt% of O-PGS.

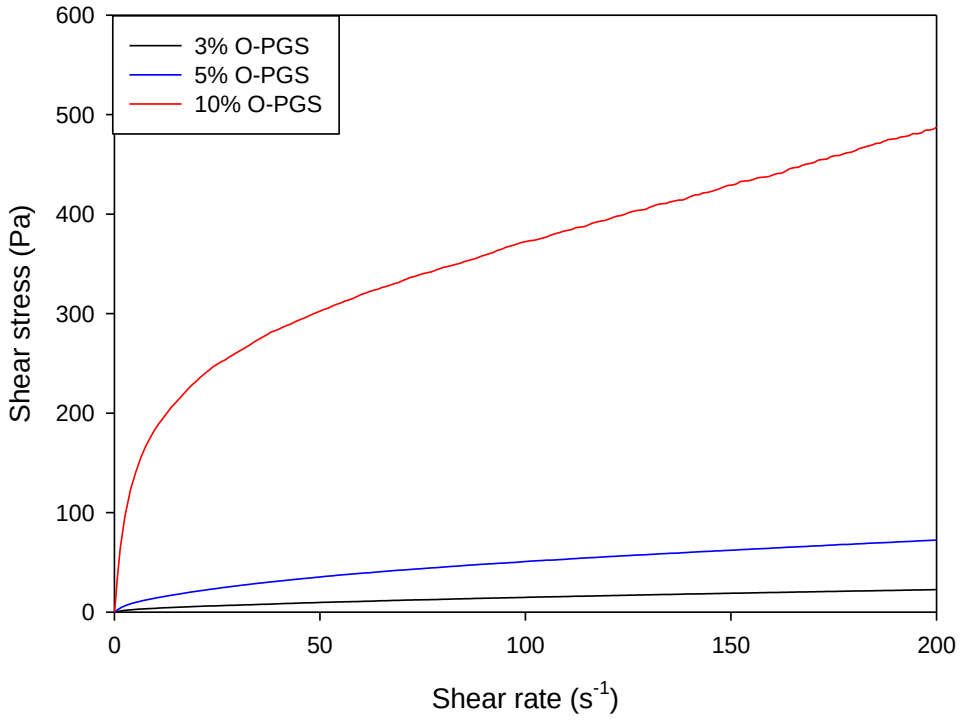

Figure 7. Representative flow curves of emulsion gels with 10 wt% oil and 3, 5, and 10 wt% of O-PGS.

Table 1. Consistency coefficient and flow behavior of emulsion gels with different O-PGS and oil concentration

O-PGS concentration (wt%)	Oil concentration (wt%)	k (Pa·s)	n (-)
3	5	0.3009±0.0086	0.6671±0.0078
	10	0.3922±0.0242	0.6666±0.0103
	20	0.9005±0.0519	0.6203±0.0059
5	5	1.0169±0.0924	0.6405±0.0134
	10	1.2950±0.1650	0.6137±0.0300
	20	4.0145±0.3779	0.5454±0.0231
10	5	6.1057±0.4836	0.6071±0.0055
	10	10.5959±0.7672	0.5792±0.0099
	20	40.7458±0.6869	0.4805±0.0068

3.3.2. Viscoelasticity of emulsion gels

Viscoelastic behavior of emulsion gels was characterized by oscillatory frequency sweep test in Figure. 5. In lower concentration of O-PGS and oil phase, emulsion showed the behavior of typical viscoelastic fluid that storage modulus and loss modulus were crossed while oscillation frequency was increasing. However, in higher concentration of O-PGS and oil phase, storage modulus was constantly higher than loss modulus in whole applied frequency range so the emulsion gel had gel-like properties.

Moreover, storage modulus and loss modulus were both increased with increasing concentration of O-PGS and oil phases. In increasing O-PGS concentration, starch gel network formed in continuous phase became denser then storage modulus and loss modulus. The relationship of starch concentration and viscoelasticity of emulsion gel was investigated in previous studies that prepared emulsion gels with mixing emulsion and starch paste.

In previous studies, gelatinized starch and emulsion gels prepared by mixing emulsion with starch paste showed similar patterns. Due to its high gel-like viscoelasticity, emulsion gels stabilized by O-PGS would be able to store lipophilic components more stably. In emulsion systems, gravitational separations, flocculation, coalescence, or Ostwald ripening can be induced by emulsion instability. In emulsion gel, higher viscosity and gel-like properties

would inhibit Brownian motion so collision frequency and velocity can be reduced so the possibility of emulsion instability can be reduced.

In many researches, oil droplets that entrapped in emulsion gels were classified into “active” and “inactive” fillers. Active fillers have mechanical interactions with continuous gel matrix while inactive fillers have no or repulsive interactions. When oil droplets were incorporated into gel matrix with octenyl succinic anhydride modified pregelatinized starch, the droplets were stabilized by amphiphilic characteristics of the starch chains. Therefore, oil droplets and continuous gel matrix had strong interactions, and the dispersed phase acted as “active filler”. Oil droplets as active filler influenced rheological properties of emulsion gels. Increase of storage and loss modulus of emulsion gels with increasing oil concentration demonstrated that oil phases acted as active filler (Dickinson, 2012). In previous studies, emulsion gels with active fillers had higher storage moduli with increasing oil contents.

Previous study which combined emulsions stabilized by whey protein isolate or Tween 20 with gelatinized rice or mung bean starch had gel-like properties of viscoelasticity (Mun et al., 2015). Another study that incorporated gelatinized wheat starch and emulsions to prepare emulsion microgel particles showed also gel-like properties that had high storage and loss modulus. However, emulsion gels prepared by whey protein isolate and

glucono-delta-lactone had decreasing storage modulus with increase of oil fraction (Rosa, Sala, Van Vliet, & Van De Velde, 2006). Moreover, emulsion gels consist of whey protein isolate and Tween 20 in aqueous phase had also reducing gel strength with increasing oil fraction (Dickinson & Chen, 1999). These results were induced by the characterization of emulsifiers and gelling agent of emulsion gels. When emulsion droplets act as an “inactive” filler in gel, the emulsifier and gel matrix has no interactions. Then, increase of oil concentration of emulsion gels induces reduced storage modulus of emulsion gels.

Moreover, many previous studies suggested that gel structures in continuous phase could affect gastrointestinal digestion pattern of oil droplets in emulsion gel. O-PGS formed gel structure in continuous phases and it disrupted invasion of lipase so lipophilic compounds that entrapped in oil droplets can be protected probably. Therefore, more investigations of digestion pattern of lipophilic compounds such as phytosterols in emulsion gels stabilized by O-PGS can be meaningful.

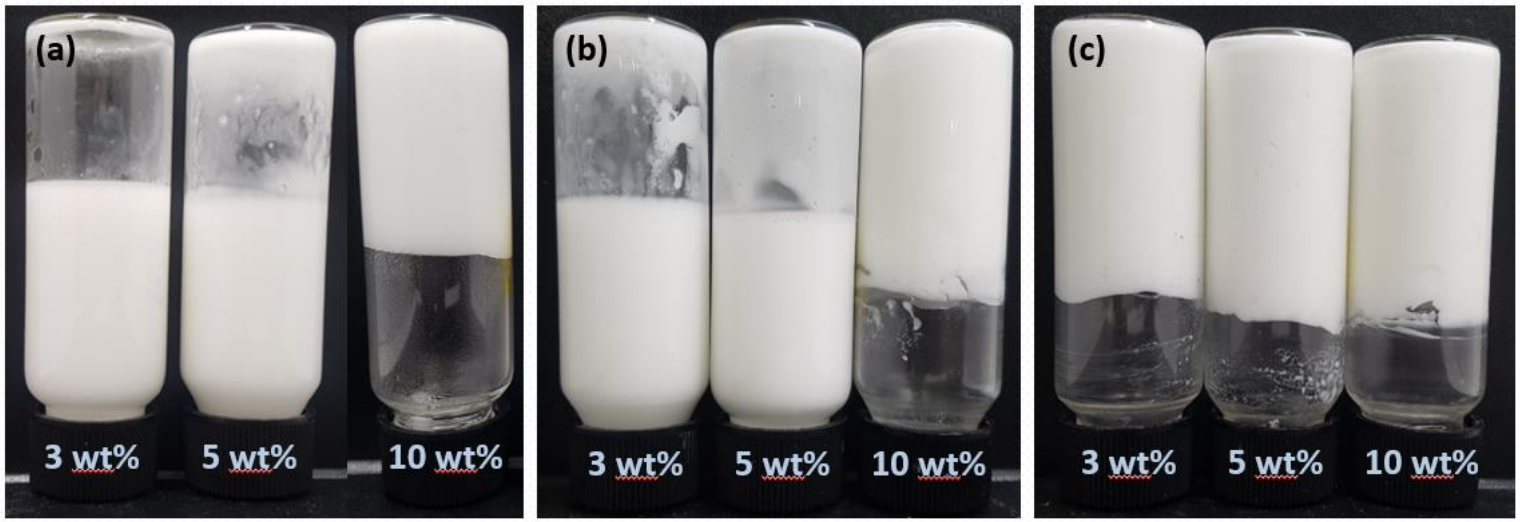

Figure 8. Photos of emulsion gels with (a) 5 wt% (b) 10 wt% (c) 20 wt% of oil phase. Letters in photo demonstrate the concentration of O-PGS used in emulsion gel preparation.

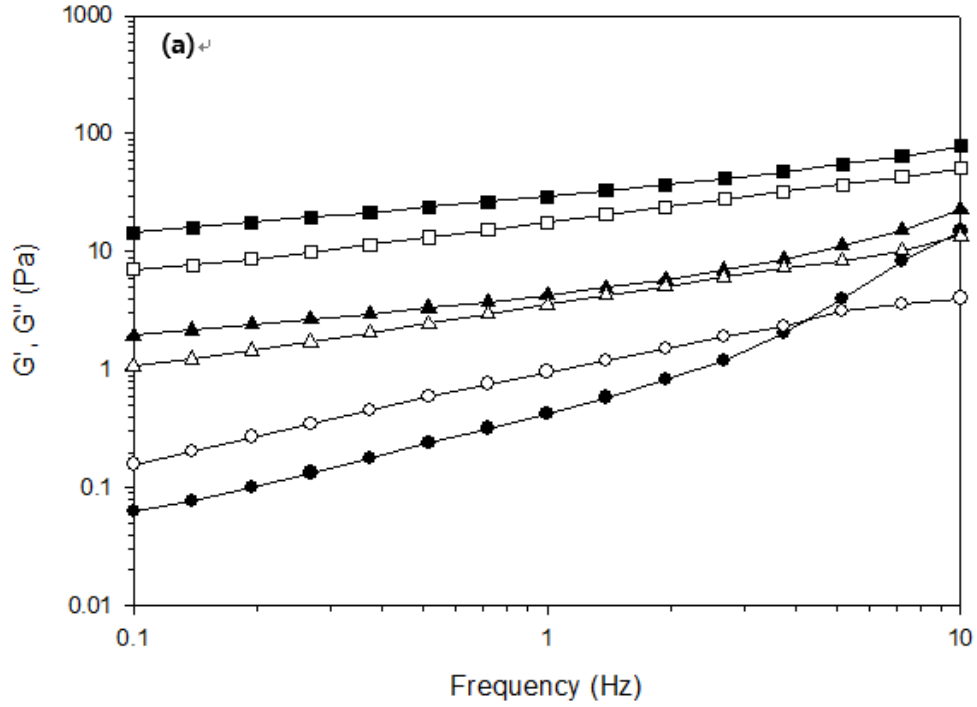

Figure 9. Rheological properties (G' and G") of emulsion gels with different O-PGS concentration and 5 wt% of oil concentration during a frequency sweep at 25°C measured by oscillatory viscoelatstic analysis.

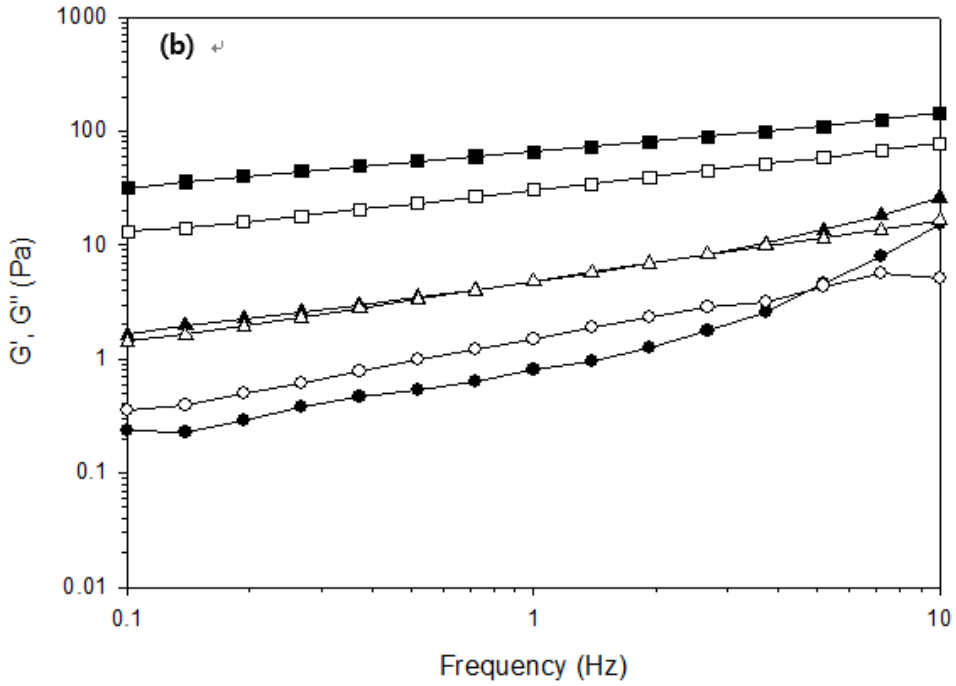

Figure 10. Rheological properties (G' and G") of emulsion gels with different O-PGS concentration and 10 wt% of oil concentration during a frequency sweep at 25℃ measured by oscillatory viscoelatstic analysis. Blank symbol represents loss modulus while filled symbol represents storage modulus. Shapes demonstrates O-PGS concentration (○, ●: 3 wt%; △, ▲: 5 wt%; □, ■: 10 wt%).

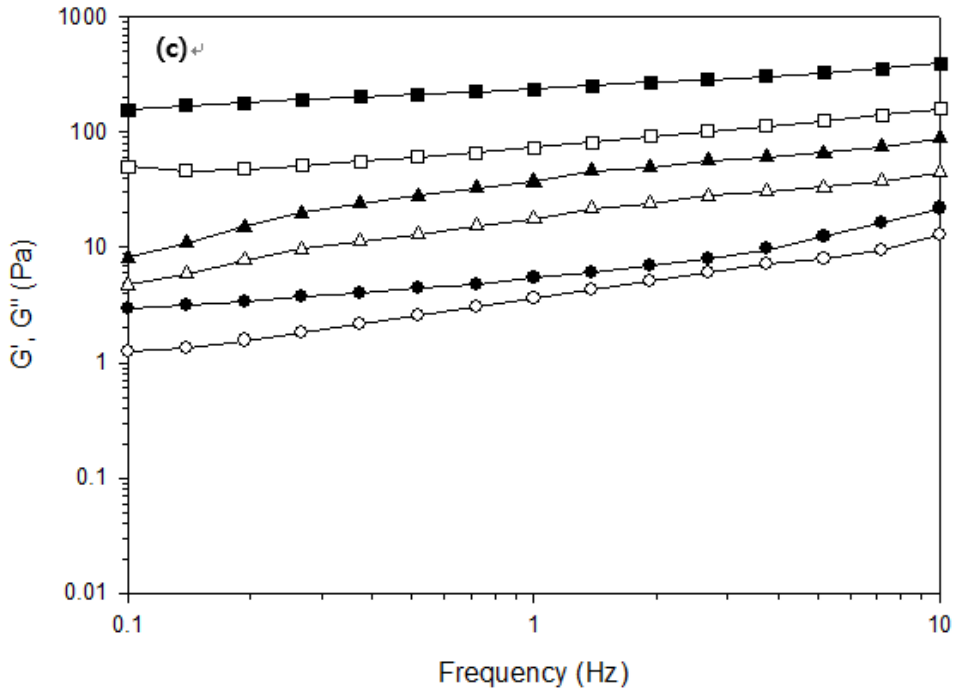

Figure 11. Rheological properties (G' and G") of emulsion gels with different O-PGS concentration and 20 wt% of oil concentration during a frequency sweep at 25℃ measured by oscillatory viscoelatstic analysis. Blank symbol represents loss modulus while filled symbol represents storage modulus. Shapes demonstrates O-PGS concentration (○, ●: 3 wt%; △, ▲: 5 wt%; □, ■: 10 wt%).

3.4. Emulsions stabilized by low concentration of O-PGS and ultrasonic treatment

3.4.1. Characteristics of emulsions

Droplet sizes and zeta-potentials of emulsions stabilized by 1.5 and 3.0 wt% of O-PGS and ultrasonic treatment were showed in Figure 12 and 13. Sonication is commonly utilized to prepare emulsions and it causes cavitation to disperse oil droplets and reduce droplet sizes (Ban, Lim, Chang, & Choi, 2014). More times of ultrasonic treatment resulted smaller droplet sizes of emulsions in Figure 12 because it provided more energies in emulsions to stabilize droplets. However, emulsions with 3.0 wt% of O-PGS showed larger droplet sizes than emulsions with 1.5 wt% of O-PGS. O-PGS forms three-dimensional starch network when dissolved in water. Many studies suggested that higher concentration of emulsifier could stabilize smaller size of emulsion droplet, but, in this study, higher concentration of O-PGS, the stabilizer, led emulsion droplets to larger sizes. Dispersed amylose and amylopectin could comprise starch network in gel structure and it could disrupt cavitation induced by ultrasonic treatment. When sonication was treated on starch paste, cavitation induced by ultrasonic could influence on both starch gel network and emulsion droplets. In many researches about the relationship between sonication and emulsion droplets resulted in increased concentration of

emulsifier led to smaller droplet sizes of emulsions (Mcclements, 2007). However, in our research, increased O-PGS concentration led to larger sized of emulsion droplets. When O-PGS concentration was higher, dispersed amylose and amylopectin comprised denser gel structure in continuous phases. Because of gel structure in continuous phase, emulsion droplets in higher concentration of O-PGS could be influenced by lower energy and cavitation of ultrasonic, so the sonication could stabilize larger sizes of droplets in higher concentration of O-PGS. In Figure 13, there was no significant difference of zeta-potential of emulsions among different O-PGS concentrations and sonication times. Zeta-potential determines electrostatic repulsion and stability of oil droplets in emulsion system. However, no quite difference of zeta-potential in different concentration of O-PGS and sonication time reveals that there was no significant difference in surface load in emulsions (Freitas & Müller, 1998).

In previous studies about the effect of ultrasonic treatment on physicochemical properties of starch paste proposed that chain scission of starch network occurred by sonication (Chang et al., 2017a, 2017b). Chain scission also led to decrease in viscosities of gel structures. In Table 2, flow behavior of emulsions is represented. Consistency coefficients of emulsions were significantly lower than emulsion gels prepared without sonication, and

it had relatively higher apparent viscosities in higher concentration of O-PGS and shorter period of ultrasonic treatment. Emulsions treated by sonication had flow behavior index around 0.95 that demonstrates it had Newtonian characteristics. Therefore, ultrasonic and cavitation reduced viscosity and pseudoplastic characteristics of emulsions.

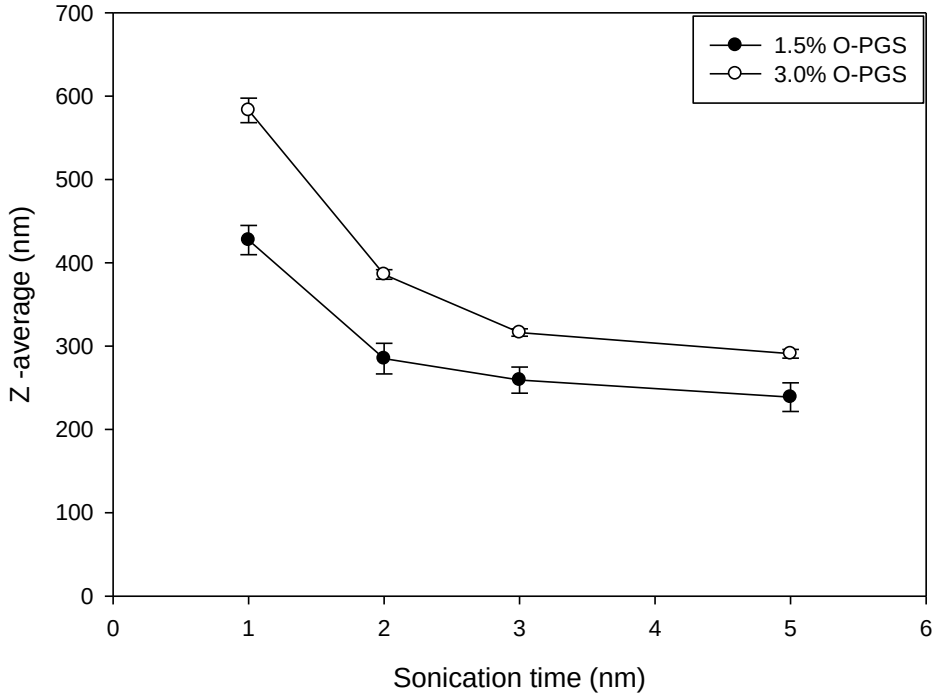

Figure 12. Droplet size (Z-average) of emulsion droplets stabilized by 1.5 and 3.0 wt% of O-PGS with different sonication times (1, 2, 3, and 5 min). Filled circles represent emulsions with 1.5 wt% of O-PGS while blank circles represent 3.0 wt% of O-PGS.

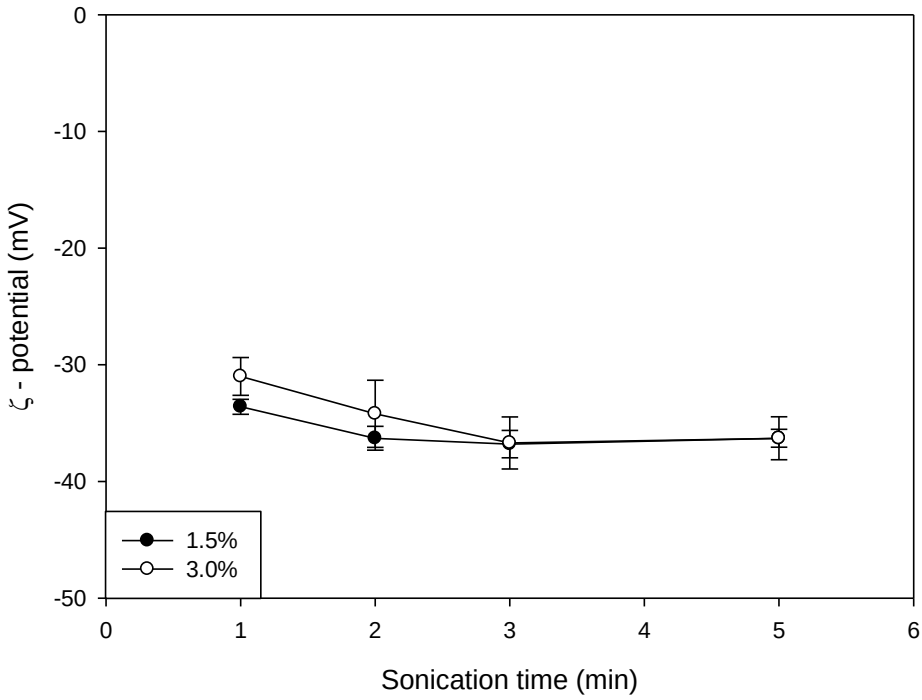

Figure 13. Zeta-potential of emulsion droplets stabilized by 1.5 and 3.0 wt% of O-PGS with different sonication times (1, 2, 3, and 5 min). Filled circles represent emulsions with 1.5 wt% of O-PGS while blank circles represent 3.0 wt% of O-PGS.

Table 2. Consistency coefficient and flow behavior of emulsions with 1.5 and 3.0 wt% of O-PGS prepared by 1,2,3, and 5 min of sonication (5 wt% of oil concentration)

O-PGS concentration (wt%)	Sonication time (min)	*k* (Pa·s)	*n* (-)
1.5	1	0.0043±0.0002	0.9278±0.0044
	2	0.0035±0.0003	0.9399±0.0025
	3	0.0032±0.0002	0.9427±0.0026
	5	0.0028±0.0001	0.9444±0.0002
3.0	1	0.0123±0.0006	0.9095±0.0059
	2	0.0078±0.0002	0.9337±0.0026
	3	0.0061±0.0001	0.9429±0.0009
	5	0.0050±0.0001	0.9478±0.0030

3.4.2. Storage stability of emulsions stabilized by O-PGS and ultrasonic treatment in different temperature

Storage stability of emulsions is one of the major part of emulsion research because of application in industrial fields. In particular, storage stability of emulsions in different temperature conditions is necessary because temperature effect on droplet size and creaming index in storage and processing (Mcclements, 2007).

Droplet sizes and zeta potentials of emulsions stabilized by 1.5 and 3.0 wt% in storage period were shown in Figure 14, 15, 16, and 17. Emulsions were stored in 4, 25, and 42℃ for 4 weeks. Basically, emulsions prepared with higher concentration of O-PGS had higher droplet sizes. However, emulsions stored in higher temperature had more increased droplet sizes during storage period while emulsions in lower temperature had less increased droplet sizes. This results told us that higher temperature storage condition led to increase in emulsion droplet sizes. In Figure 15 and 16, zeta-potential of emulsions were not significantly influenced by storage temperature, but in higher temperature the absolute value of zeta-potentials was slightly decreased.

In emulsion stability, emulsion coalescence and flocculation are the major phenomenon of instability. Emulsion coalescence is occurring when the interfaces of emulsions were broken by collision between emulsions.

Therefore, collision frequency and emulsion velocity affected significantly on droplet sizes in different storage temperatures.

In higher temperature, emulsion droplets had higher velocity and collision frequency. Therefore, emulsion droplets had larger diameter in higher temperature. However, as shown in the zeta-potentials of emulsions were in the range of -30 - -40 mV. In this range of zeta-potential, emulsion flocculation was hard to occur.

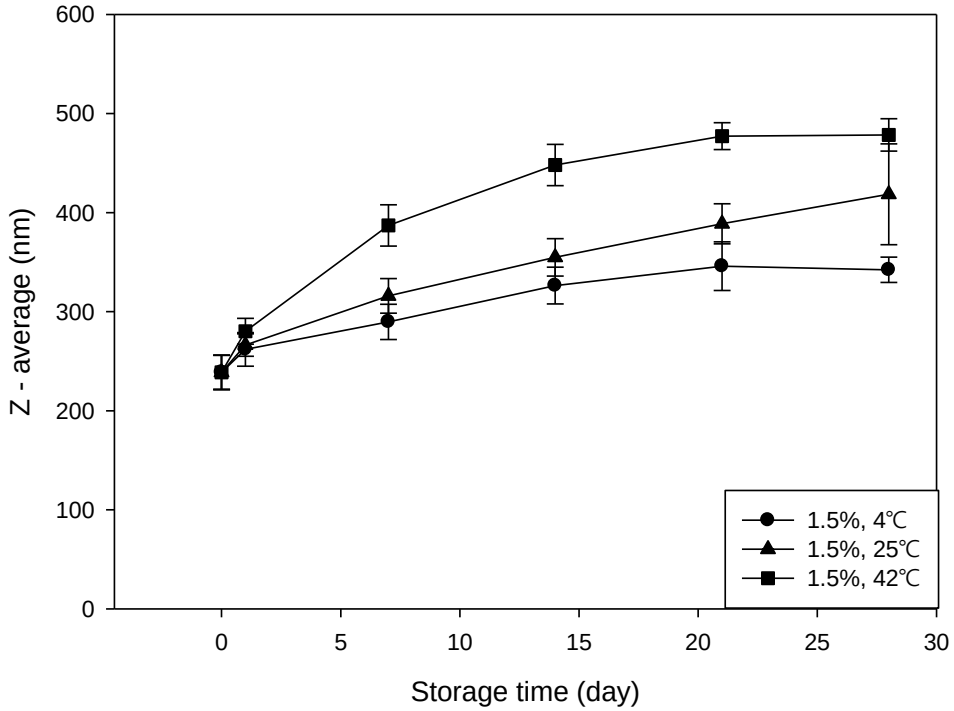

Figure 14. Droplet size (Z-average) of emulsion droplets stabilized by 1.5 wt% of O-PGS during 4 weeks of storage in different temperature conditions.

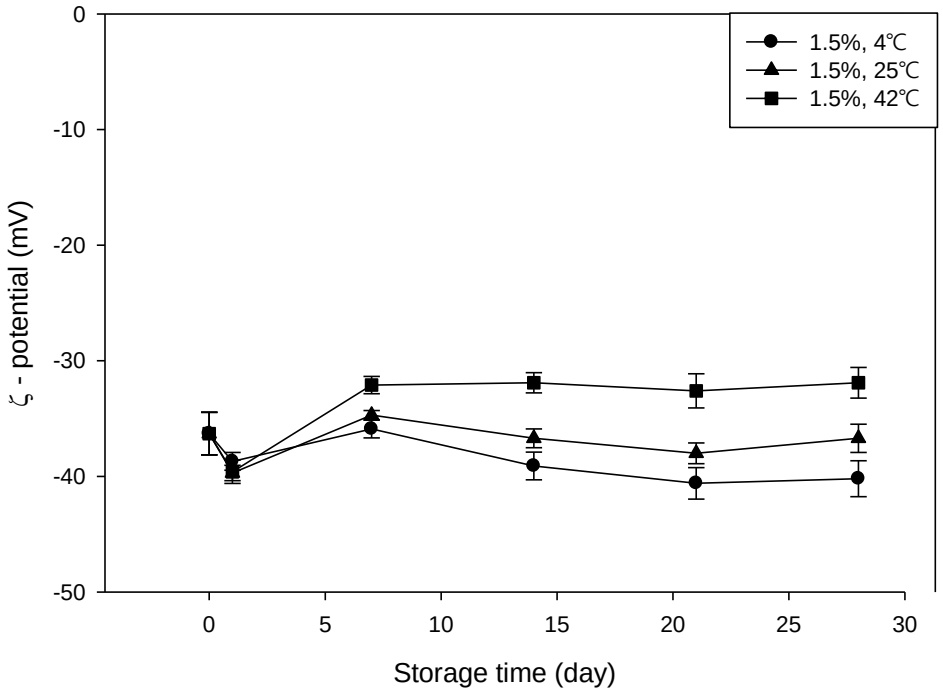

Figure 15. Zeta-potential of emulsion droplets stabilized by 1.5 wt% of O-PGS during 4 weeks of storage in different temperature conditions.

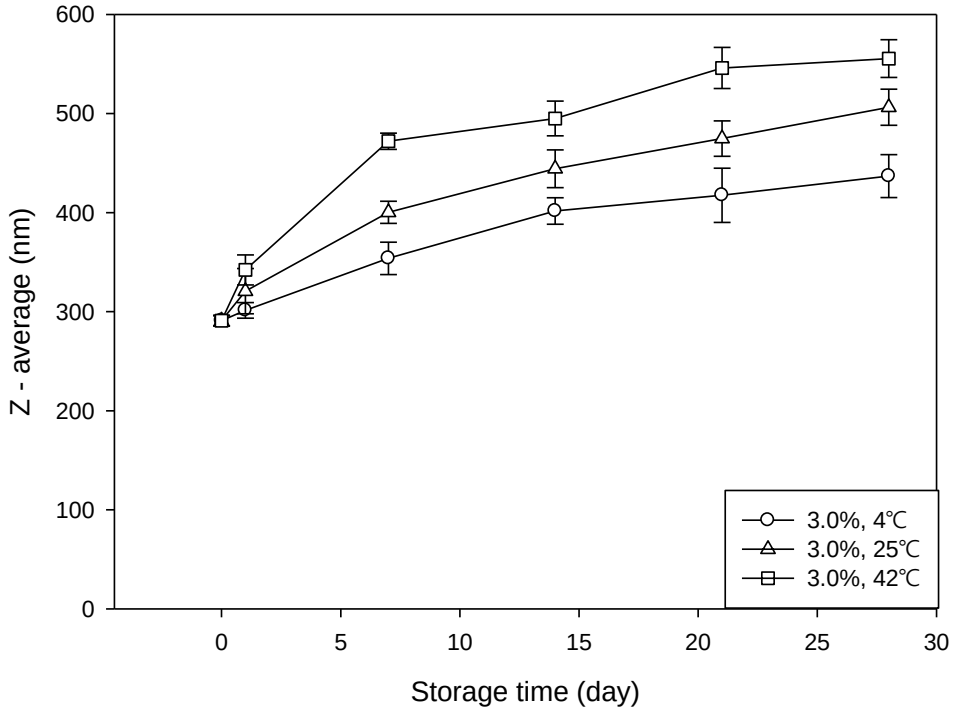

Figure 16. Droplet size (Z-average) of emulsion droplets stabilized by 3.0 wt% of O-PGS during 4 weeks of storage in different temperature conditions.

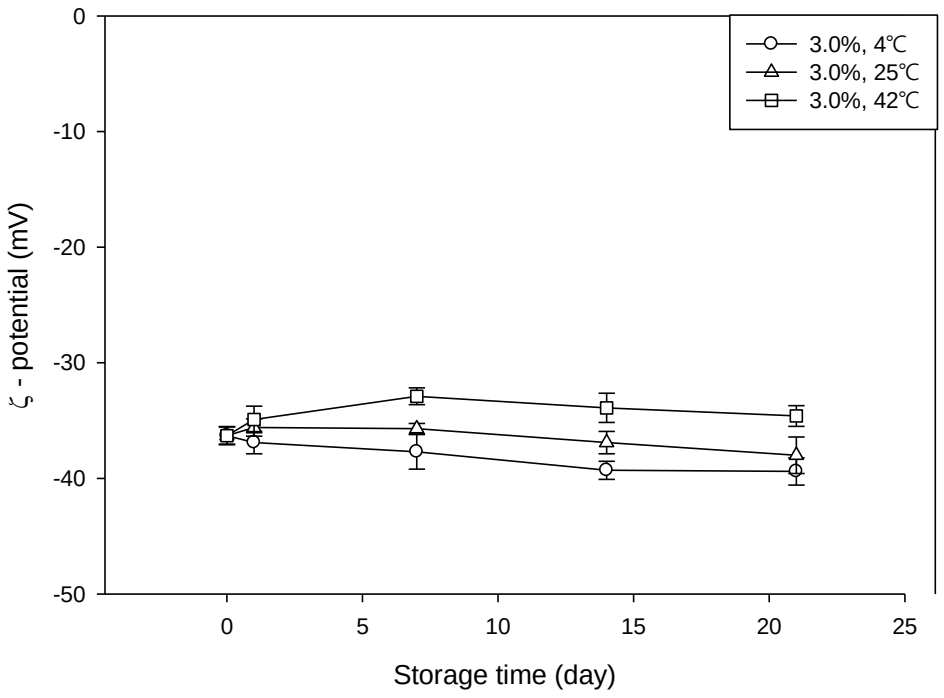

Figure 17. Zeta-potential of emulsion droplets stabilized by 3.0 wt% of O-PGS during 4 weeks of storage in different temperature conditions.

Ⅳ. CONCLUSIONS

In this study, preparation of O-PGS collapsed A-type crystalline structure of original standard maize starch granules, and ethanol precipitation induced V-type crystal structure in powdered PGS and O-PGS. The addition of OSA had no effects on crystalline structure of PGS. DSC data showed that normal maize starch started gelatinization at about 65℃, and O-PGS and PGS prepared by ethanol precipitation was cold-water soluble. OSA-modification of starch paste was carried out successfully and it maintained during precipitation and freeze drying step. OSA-modification increased hydrophobicity of PGS and it could stabilize oil-water interfaces and formed emulsion gels that contain and stabilize oil droplets in continuous gel matrix. The emulsion gels had typical pseudoplastic behavior in increasing shear rate. Increase in O-PGS and oil concentration led to increase in apparent viscosities and pseudoplastic characteristics of emulsion gels due to increasing density of starch network in gel phase and the droplets acted as active filler in emulsion gels. Storage and loss moduli of emulsion gels increased with increasing O-PGS and oil concentration. When O-PGS and oil concentration was high enough, storage modulus was higher than loss modulus in frequency range, then it had gel-like properties. Ultrasonic treatment reduced droplet size of emulsions and starch network in continuous phase disrupted the effect of

sonication in decreasing droplet sizes. Emulsion droplets showed constant zeta-potential in storage and droplet sizes increased more in higher temperature of storage. Emulsion gels stabilized by physically modified OSA-starch did not need any more additional substances or processes, so it can be expected to be applied in various fields in foods, cosmetics, and pharmaceuticals.

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국 문 초 록

이 연구의 목적은 옥테닐호박산이 치환된 알파 전분을 제조하고 그 물리화학적 특성을 확인하며 이를 통해 안정화 된 에멀션 젤의 특성을 파악하는데 있다. 옥테닐호박산이 치환된 알파 전분을 제조하기 위하여 옥수수 전분 수용액을 가열하여 호화시키고 옥테닐호박산 치환 반응을 일으킨 후 에탄올을 활용한 침전 및 동결건조를 수행하였다. 에탄올 침전에 의한 알파 전분의 결정 구조는 이전의 옥수수 전분과는 크게 다른 구조를 갖고 있으며, 옥테닐호박산 치환 반응은 알파 전분의 결정 구조에 큰 영향을 끼치지 않았다. 시차열량주사계를 통해 알파 전분과 옥테닐호박산이 치환된 알파 전분이 차가운 물에서도 녹는 것을 확인했고, 푸리에 변환 적외분광분석을 통해 옥테닐호박산의 치환 반응이 성공적으로 일어났으며, 에탄올 침전 및 동결 건조 단계를 거치는 동안 유지되었음을 확인했다. 이를 통해 에멀션 젤을 제조시 기름과 물의 계면이 옥테닐호박산이 치환된 알파 전분에 의해 안정화 되었고 기름 방울이 젤로 구성된 연속상 내부에 둘러싸인 것을 확인했다. 에멀션 젤은 전형적인 유사가소성 거동을 보였다. 옥테닐호박산이 치환된 알파 전분 및 기름의 농도가 높을수록 에멀션 젤의 겉보기 점도와 가소성 특성이 증가했다. 점탄성을 확인할 시 에멀션 젤은 옥테닐호박산이 치환된 알파 전분과 기름의 농도를 증가시킴으로써 더 높은 저장탄성률과 손실탄성률을 나타내었다. 즉, 서로 다른 농도의 유상 및 옥테닐호박산이 치환된 알파 전분에 의해 제조 된 에멀션 젤은 농도에 따라 광범위한 유변학적 특성을 나타내었다. 결론적으로, 옥테닐

호박산이 치환된 알파 전분은 광범위한 물성을 지닌 식품을 제조할 때 또는 친유성을 가진 기능성 물질을 식품 내 포집할 때 활용될 수 있다.

주요어: 에멀션 젤, 옥테닐호박산 전분, 알파 전분, 하이드로젤, 에멀션 유변학

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